Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

Claims 1-8 (Cancelled).

9(Original). A method for detection of differential expression of natural antisense messenger RNA (mRNA), comprising:

(a) separately obtaining polyA-mRNA-A molecules from cell population A and polyA-mRNA-B molecules from cell population B;

(b) separately generating by a reverse transcription enzyme a population of single-stranded cDNA-A molecules from polyA-mRNA-A and a population of single-stranded cDNA-B molecules from polyA-mRNA-B, wherein the polydeoxythymidine containing oligonucleotide primer used to produce the cDNA-B molecules comprises a specific bacteriophage RNA polymerase promoter region close to its 5' terminus;

(c) incubating the combined populations of single-stranded cDNA-A molecules and single-stranded cDNA-B molecules, under conditions allowing hybridization of sense cDNA molecules with antisense cDNA molecules, wherein each single-stranded antisense cDNA molecule that hybridizes has a segment complementary to the sense DNA molecule and hybridizes thereto to form a hybrid molecule with a double-stranded segment;

(d) treating the hybrid molecules with a DNA polymerase having a 5′ to 3′ polymerase activity and a 3′ to 5′ exonuclease activity to remove single-stranded non-hybridized segments of the hybrid molecule from 3′ to 5′ and to extend the double-stranded segment of the hybrid molecule 5′ to 3′ over an adjacent single-stranded segment as template, thereby forming a double-stranded molecule having the RNA polymerase promoter region close to one terminus;

(e) using the double-stranded molecule as a template for the specific RNA polymerase to produce a population of RNA molecules;

(f) labeling with a first label the RNA molecules produced in step (e);

(g) labeling with a second label as control the polyA-mRNA-A molecules and/or the polyA-mRNA-B molecules of step (a);

(h) mixing labeled RNA molecules from steps (f) and (g) and hybridizing them to a DNA microarray; and

(i) identifying the genes on the microarray which are preferentially labeled with the labeled RNA molecules of step (f).

10(Currently amended). The method according to claim [[10]] 9, wherein the specifc bacteriophage polymerase is selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase, and SP6 RNA polymerase.

11(Currently amended). The method according to claim [[10]] 9, wherein following step (b) the cDNA-B is modified in order to resist the 3' to 5' exonuclease activity of step (d).

12(Currently amended). The method according to claim [[12]] 11, wherein in step (d) the 3' terminus of the cDNA-B is modified.

13(Currently amended). The method according to claim [[12]] 11, wherein in step (d) the entire cDNA-B is modified.

14(Currently amended). The method according to claim [[13]] 12, wherein 3' terminus of the cDNA-B is modified by addition of a nucleotide analog.

15(Currently amended). The method according to claim [[14]] 13, wherein the entire cDNA-B is modified by incorporation of nucleotide analogs.

16(Currently amended). The method according to claim [[10]] 9, wherein the first label of step (f) is Cy3, and the second label of step (g) is Cy5.

17(Currently amended). The method according to claim [[10]] 9, wherein the first label of step (f) is Cy5, and the second label of step (g) is Cy3.

18(Original). A method for detection of differential expression of natural antisense messenger RNA (mRNA), comprising:

(a) separately obtaining polyA-mRNA-A molecules from cell population A and polyA-mRNA-B molecules from cell population B;

(b) separately generating by a reverse transcription enzyme a population of single-stranded cDNA-A molecules from polyA-mRNA-A and a population of single-stranded cDNA-B molecules from polyA-mRNA-B, wherein the polydeoxythymidine containing oligonucleotide primer used to produce the cDNA-A molecules comprises close to its 5′ terminus a sequence identical to an amplification primer used in step (e) and wherein the polydeoxythymidine containing oligonucleotide primer used to produce the cDNA-B molecules comprises a specific bacteriophage RNA polymerase promoter region close to its 5′ terminus;

(c) incubating the combined populations of single-stranded cDNA-A molecules and single-stranded cDNA-B molecules, under conditions allowing hybridization of sense cDNA molecules with antisense cDNA molecules, wherein each single-stranded antisense cDNA molecule that hybridizes has a segment complementary to the sense DNA molecule and hybridizes thereto to form a hybrid molecule with a double-stranded segment;

(d) treating the hybrid molecules with a DNA polymerase having a 5′ to 3′ polymerase activity and a 3′ to 5′ exonuclease activity to remove single-stranded non-hybridized segments of the hybrid molecule from 3′ to 5′ and to extend the double-

stranded segment of the hybrid molecule 5' to 3' over an adjacent single-stranded segment as template, thereby forming a double-stranded molecule having the RNA polymerase promoter region close to one terminus;

(e) amplifying the double-stranded molecule of step (d) using a thermostable polymerase and a first amplification primer identical to the sequence used in step (b) and a second amplification primer identical to the specific bacteriophage RNA polymerase promoter region of step (b);

(f) using the double-stranded molecules so produced as a template for the specific RNA polymerase to produce a population of RNA molecules;

(g) labeling with a first label the RNA molecules produced in step (f);

(h) labeling with a second label as control the polyA-mRNA-A molecules and/or the polyA-mRNA-B molecules of step (a);

(i) mixing labeled RNA molecules from steps (g) and (h) and hybridizing them to a DNA microarray; and

(i) identifying the genes on the microarray which are preferentially labeled with the labeled RNA molecules of step (g).

19(Currently amended). The method according to claim [[19]] 18, wherein the specifc bacteriophage polymerase is

selected from the group consisting of T7 RNA polymerase, T3 RNA polymerase, and SP6 RNA polymerase.

20(Currently amended). The method according to claim [[19]] 18, wherein following step (b) the cDNA-B is modified in order to resist the 3' to 5' exonuclease activity of step (d).

21(Currently amended). The method according to claim [[21]] 20, wherein in step (d) the 3' terminus of the cDNA-B is modified.

22(Currently amended). The method according to claim [[21]] 20, wherein in step (d) the entire cDNA-B is modified.

23(Currently amended). The method according to claim [[22]] 21, wherein 3' terminus of the cDNA-B is modified by addition of a nucleotide analog.

24(Currently amended). The method according to claim [[23]] 22, wherein the entire cDNA-B is modified by incorporation of nucleotide analogs.

25(Currently amended). The method according to claim [[19]] 18, wherein the first label of step (g) is Cy3, and the second label of step (h) is Cy5.

26(Currently amended). The method according to claim [[10]] 9, wherein the first label of step (g) is Cy5, and the second label of step (h) is Cy3.